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Effect of Anoxia on the Responsiveness of the Adrenal Medulla to Splanchnic Nerve Stimulation

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In dogs anesthetized with sodium pentobarbital, the adrenal venous blood was collected and was estimated for adrenaline by the arseno-molybdic acid method. Simultaneously, arterial blood samples were taken and the blood gas contents were estimated by the manometric method. Anoxia was induced by inhalation of 5.7－7.2 per cent oxygen in nitrogen or 4.4－5.8 per cent oxygen and 1.1－6.7 per cent carbon dioxide in nitrogen. The adrenal medullary response to splanchnic nerve stimulation during low oxygen air inhalation was always found to be greater than during normal air inhalation. It is concluded that the responsiveness of the adrenal medulla to splanchnic nerve stimulation is increased by lack of oxygen.

It has been well established that the adrenaline secretion rate of the adrenal gland is markedly increased by splanchnic nerve stimulation. On the other hand, BROENING[2] found that oxygen consumption rate of the adrenal gland was definitely increased by splanchnic nerve stimulation. Thus, it is assumed that augmentation of adrenaline secretion caused by splanchnic nerve stimulation depends largely upon the oxidation process in the adrenal medulla. From this viewpoint it is of interest to know how various kinds of anoxia affect the responsiveness of the adrenal medulla to splanchnic nerve stimulation.

In experiments with the perfused adrenal gland, SZENTJURIN[4] was unable to observe any definite inhibitory effect of cyanide anoxia upon the responsiveness of the adrenal gland to splanchnic nerve stimulation.

The present investigation was designed to ascertain the effect of anoxic anoxia produced by inhalation of low oxygen air upon the responsiveness of the adrenal medulla to splanchnic nerve stimulation.

EXPERIMENTAL METHODS

Seventeen mongrel dogs, 5.8－14.7 kg in weight, were used in the experiments. These dogs were anesthetized by intravenous injection of sodium pentobarbital (Nembutal).

The adrenal vein was exposed through the lumbar route and a small cannula for collecting the adrenal venous blood was inserted into the

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vein lateral to the adrenal gland[3]. In most experiments the blood pressure was recorded by a mercury manometer connected with the femoral artery.

Gas mixtures of 5.7–7.2 per cent oxygen in nitrogen or 4.4–5 8 per cent oxygen and 1.1–6.7 per cent carbon dioxide in nitrogen were previously prepared and stored in the Douglas bag. Analysis of the gas mixtures was made using the Haldane gas-analytic apparatus. In order to produce an anoxic condition, about 150 ℓ of the above-mentioned air with low oxygen content were inhaled by the animal through a tracheal cannula.

The splanchnic nerves were cut at a point below the diaphragm and the peripheral parts were stimulated electrically with the square wave current of 17 V, 50 c/s and 2 msec. puls duration for 30 seconds using an electronic stimulator (Nikkoh MSE-2 stimulator with MSE-2-JA isolator).

In the main experiments, the stimulation was performed before, during, and after inhalation of air with low oxygen content.

In control experiments the stimulation was applied three times during normal air inhalation.

To measure the blood gas content, blood samples were taken with a syringe from the femoral artery and immediately poured into a test tube containing sodium fluorate and potassium oxalate covered with liquid paraffin. Oxygen and carbon dioxide contents of arterial blood samples were determined by the manometric method of VAN SLYKE and NEILL[5].

The adrenal venous blood was collected before splanchnic nerve stimulation. It was also collected continuously during the first and second 60-second periods after the onset of stimulation. The adrenaline content of the adrenal venous blood specimens was determined colorimetrically following the method of BLOOR and BULLEN[1], adrenaline (Sankyo Co.) being used as the standard.

RESULTS AND DISCUSSION

Control experiments

Under normal breathing condition, splanchnic nerve stimulation was applied three times at intervals of 15 minutes. Immediately after initiation of stimulation, blood pressure rose steeply, showing two peaks. After cessation of stimulation, it decreased gradually.

The rate of adrenaline secretion before each splanchnic nerve stimulation was smaller than 0.04 μg/kg/min. in all cases except one, in which 0.07 μg were measured. Maximum augmentation of adrenaline secretion was observed in the first 60-second period after the start of splanchnic nerve stimulation.

In Exp. 1, the adrenaline secretion rate was measured as 0.20, 0.11, and 0.24 μg/kg/min. after the first, second, and third stimulations, respectively. The secretion rate after the second stimulation was

in this case somewhat smaller than after the first and third stimulations.

In Exp. 2, the rate of adrenaline secretion in the first 60-second period after the start of stimulation was measured as 0.25, 0.28, and 0.26 μg/kg/min. in the first, second, and third stimulations, respectively.

In Exp. 3, the adrenaline secretion rate in the first 60-second period after the first and third stimulations was almost the same and was measured as 0.15 and 0.14 μg/kg/min., respectively. The rate of adrenaline secretion after the second stimulation was somewhat higher than others, 0.21 μg being estimated.

In Exp. 4, a remarkably augmented adrenaline secretion, such as 0.89, 0.97, and 0.75 μg/kg/min., was observed in the first 60-second period after the first, second, and third splanchnic nerve stimulations, respectively.

In Exp. 5, splanchnic nerve stimulation was applied twice. Adrenaline secretion rate after the first and second splanchnic nerve stimulations was 0.41 and 0.43 μg/kg/min.

In Exps. 1–5, the adrenaline secretion rate after the second and third splanchnic nerve stimulations expressed as per cent of the secretion rate after the first stimulation, was 104±12% (mean±standard error of the mean) and 100±7%, respectively (Table 1.). Thus it is concluded that the adrenaline secretory response of the same adrenal gland to each splanchnic nerve stimulation does not vary under the same experimental conditions.

Anoxia experiments

Inhalation of 5.7–7.2 per cent oxygen in nitrogen was performed on six dogs. Soon after the start of hypoxic air inhalation, the rate of respiration increased to two to three times as much as before inhalation. However, there was no noticeable alteration in intensity of respiration. The blood oxygen and carbon dioxide contents were decreased by hypoxic air inhalation. Before inhalation, they were measured as 15.8 (12.0–20.3) vol. per cent for oxygen and 44 0 (36.9–48.6) vol. per cent for carbon dioxide. From 8 to 15 minutes after the initiation of inhalation when the second splanchnic nerve stimulation was performed, they were estimated as 9.2 (7.1–11.7) vol. per cent for oxygen and 36.5 (29.7–41.2) vol. per cent for carbon dioxide. Just after the termination of inhalation, breathing of ordinary air was resumed. The blood oxygen and carbon dioxide contents increased again and when the third splanchnic nerve stimulation was performed after 27–38 minutes, they were estimated as 18.0 (14.0–23.3) vol. per cent for oxygen and 38.5 (30.9–43.7) vol. per cent for carbon dioxide. The blood pressure fluctuation after splanchnic nerve stimulation in these experiments was almost the same as that in control experiments.

The first, second, and third splanchnic nerve stimulations, which were applied before, during, and after hypoxic air inhalation, induced a

definite increase in the adrenaline secretion rate. Maximal increase was always observed in the first 60-second period after stimulation. In the second 60-second period, the secretion rate was almost the same as or somewhat higher than the pre-stimulation secretion rate.

The adrenaline secretion rate before the first and third splanchnic nerve stimulations was less than 0.07 μg/kg/min., showing no significant difference from control experiments. The rate of adrenaline secretion before the second stimulation was measured as 0.03−0.16 μg.

In Exp. 6, the maximal adrenaline secretion rate after the first, second, and third splanchnic nerve stimulations was 0.45, 0.63, and 0.23 μg/kg/min., respectively.

In Exp. 7, the adrenaline secretion rate increased after the first and third splanchnic nerve stimulations to 0.49 and 0.85 μg/kg/min., respectively. After the second stimulation, it increased to 1.2 μg.

Similar results were obtained in Exp. 8. The maximum rate of adrenaline secretion after the first, second, and third splanchnic nerve stimulations was 0.35, 0.99, and 0.44 μg/kg/min., respectively.

The results of Exp. 9 were almost the same as those of Exps. 7 and 8. The maximum adrenaline secretion rate after the first, second, and third stimulations was 0.56, 1.0, and 0.48 μg/kg/min., respectively.

In Exp. 10, the adrenaline secretion rate increased to 0.30 and 0.36 μg/kg/min. after the first and third splanchnic nerve stimulations, respectively. After the second stimulation it increased to 0.41 μg.

In Exp. 11, the maximum rate of adrenaline secretion after the first and third splanchnic nerve stimulations was 0.15 and 0.09 μg/kg/min., respectively. After the second stimulation, it was 0.91 μg.

In Exps. 6−11, in which inhalation of 5.7−7.2 per cent oxygen in nitrogen was performed, a decrease in blood carbon dioxide content as well as in blood oxygen content was observed. In these cases there is a possibility that hypocapnia itself will affect the responsiveness of the adrenal medulla to splanchnic nerve stimulation. In order to exclude this possibility, gas mixtures of 5.7−5.8 per cent oxygen and 1.1−1.5 per cent carbon dioxide in nitrogen were prepared and inhaled by two dogs (Exps. 12 and 13).

After inhalation, respiration became faster and deeper. Blood oxygen and carbon dioxide contents before inhalation were measured as 17.2 (16.8−17.6) vol. per cent for oxygen and 51.4−51.5 vol. per cent for carbon dioxide. From 14 to 19 minutes after the initiation of inhalation when the second splanchnic nerve stimulation was applied, they were 11.0 (10.1−11.9) vol. per cent for oxygen and 44.3 (42.5−46.0) vol. per cent for carbon dioxide. Thus the blood carbon dioxide content during hypoxic air inhalation was found in these experiments to be smaller than before inhalation. When the third stimulation was applied 28 to 32 minutes after termination of inhalation, they were measured as 19.8

(17.7−21.8) vol. per cent for oxygen and 45.7 (43.7 − 47.7) vol. per cent for carbon dioxide. Similar alteration in blood pressure after splanchnic nerve stimulation to that in control experiments and Exps. 6−11 was found in Exps. 12−13.

In Exp. 12, the adrenaline secretion rate before the first and third splanchnic nerve stimulations was immeasurably small. It increased to 0.37 and 0.98 μg/kg/min. by the first and third splanchnic nerve stimulations. The adrenaline secretion rate before the second splanchnic nerve stimulation was higher than the pre-stimulation secretion rate of the control experiments and was measured as 0.22 μg. It was increased markedly by the second splanchnic nerve stimulation and reached 2.2 μg.

In Exp. 13, the adrenaline secretion rate before the splanchnic nerve stimulations was 0.07−0.10 μg/kg/min. It was augmented by the first, second, and third splanchnic nerve stimulations. The maximum secretion rate after stimulation was measured as 0.22, 0.43, and 0.36 μg, respectively.

In Exps. 12 and 13 described above, there was a slight decrease in blood carbon dioxide content during hypoxic inhalation. For the purpose of complete exclusion of the possible effect of hypocapnia upon the responsiveness of the adrenal medulla to splanchnic nerve stimulation, a further attempt was made to perform experiments on dogs in which there was only decrease in blood oxygen content and no definite alteration in blood carbon dioxide content. Gas mixtures of 4.4−5.5 per cent oxygen and 6.1−6.7 per cent carbon dioxide in nitrogen were prepared and inhaled. Experiments were performed on four dogs (Exps. 14−17). Just after the initiation of inhalation, respiration became deeper and faster. The blood pressure elevation elicited by splanchnic nerve stimulation in these experiments was almost the same as that in Exps. 6−11 and Exps. 12−13. The blood oxygen content before inhalation was 16.9 (13.6−20.3) vol. per cent and that of carbon dioxide was 52.0 (44.6−67.3) vol. per cent. After inhalation the oxygen content of the blood decreased markedly, whereas the carbon dioxide content did not show any definite alteration. Six to 11 minutes after the start of inhalation, when the second splanchnic nerve stimulation was applied, the blood oxygen content was measured as 6.3 (3.6−9.4) vol. per cent and the blood carbon dioxide content was determined as 50.0 (45.7−57.2) vol. per cent. Thirty to 43 minutes after the termination of inhalation, the estimates were 19.3 (13.2−24.7) vol. per cent for oxygen and 39.5 (33.9−49.0) vol. per cent for carbon dioxide.

The adrenaline secretion rate before the first and third splanchnic nerve stimulations was less than 0.04 μg/kg/min. and that before the second stimulation was 0.07−0.09 μg in three out of four cases. In the other case (Exp. 15), it was measured as 0.21 μg.

By splanchnic nerve stimulation, the adrenaline secretion rate increased without exception and the maximum increase was always observed

Table 1.
Effect of Anoxia Induced by the Low Oxygen Air Inhalation upon the Responsiveness of the Adrenal Medulla to the Splanchnic Nerve Stimulation

No. of Expt.	Low oxygen air	Adrenaline secretion rate after the splanchnic nerve stimulation, expressed as per cent of the pre-inhalation rate		
		Before inhalation	During inhalation	After inhalation
1	Control expts.	100	55	120
2		100	112	104
3		100	140	93
4		100	109	84
5		100	105	
	Mean ± S.E.	100	104 ± 12	100 ± 7
6	5.7-7.2% O_2 in N_2	100	140	51
7		100	245	173
8		100	283	126
9		100	179	86
10		100	137	120
11		100	607	60
	Mean ± S.E.	100	265 ± 66	103 ± 17
12	5.7-5.8% O_2 and 1.1-1.5% CO_2 in N_2	100	595	265
13		100	195	164
	Mean ± S.E.	100	395 ± 141	215 ± 36
14	4.4-5.5% O_2 and 6.1-6.7% CO_2 in N_2	100	240	47
15		100	373	109
16		100	436	236
17		100	231	161
	Mean ± S.E.	100	320 ± 44	138 ± 35

in the first 60-second period after stimulation.

In Exp. 14, the maximum rate of adrenaline secretion after the first, second, and third splanchnic nerve stimulations was 0.15, 0.36, and 0.07 μg/kg/min., respectively.

In Exp. 15, the adrenaline secretion rate increased and reached 0.22 and 0.24 μg/kg/min. after the first and third splanchnic nerve stimulations. After the second stimulation it was increased to 0.82 μg.

In Exp. 16, the adrenaline secretion rate in the first 60-second period after the first, second, and third splanchnic nerve stimulations was measured as 0.11, 0.48, and 0.26 μg/kg/min., respectively.

In Exp. 17, the rate of adrenaline secretion was increased by splanchnic nerve stimulation, 0.36, 0.83, and 0.53 μg/kg/min. being measured after the first, second, and third stimulations.

In order to compare the adrenaline secretory response of the adrenal medulla to splanchnic nerve stimulation under anoxic conditions with that under normal ones, the maximum adrenaline secretion rate after the second splanchnic nerve stimulation (during hypoxic air inhalation) was expressed as a percentage of the secretion rate after the first stimulation (before inhalation). It was 265±66% in Exps. 6–11, 395±141% in Exps. 12–13, and 320±44% in Exps. 14–17 (Table 1.). Thus the adrenaline secretion rate after splanchnic nerve stimulation under anoxic conditions was found to be always greater than that under normal conditions. It is assumed that the responsiveness of the adrenal medulla to splanchnic nerve stimulation is definitely increased by lack of oxygen supply to the adrenal gland.

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